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THE ENZYME PROBLEM AND BIOLOGICAL OXIDATIONS[1]

PROF. OTTO WARBURG

Kaiser Wilhelm Institut, Berlin-Dahlem

In 1820 Edmund Davy discovered that ethyl alcohol when passed over platinum black in the presence of molecular oxygen is oxidized to acetic acid. This experiment caused a great sensation. Alcohol, which is otherwise stable in the presence of molecular oxygen, here was oxidized to acetic acid at as low a temperature as in acetic acid bacteria. Berzelius, under the influence of Davy's experiment, wrote: "The fermentations are possibly brought about by forces like those brought about by platinum black." For Schönbein the catalytic action of platinum black was the prototype of all fermentations. Bredig called the finely divided platinum metals inorganic enzymes.

With the development of organic chemistry Davy's experiment lost much of its interest. The similarity of action of both platinum black and enzymes was no longer taken too seriously. Platinum was inorganic, the enzymes belonged to organic chemistry.

I. NEWER DEVELOPMENT

The newer development of the enzyme problem began with a chance discovery. In experiments on the respiration of sea-urchin's eggs, the production of carbon dioxide was determined by displacing it with tartaric acid. It was then noted that tartaric acid, when in contact with the egg substance, was rapidly oxidized by the oxygen of the air. The cell substance therefore contained a catalyst which caused the oxidation of the otherwise stable tartaric acid. This catalyst proved to be resistant to boiling, even resistant to incandescence, and was nothing else than iron which occurs in traces in the sea-urchin's egg.

Attention was thus called to iron, and work was undertaken to investigate whether physiological oxidation had any relation to iron.

[1] Herter Lecture delivered at the Johns Hopkins University School of Medicine, October 19, 1929.

It was found that traces of iron added to the sea urchin's egg substance accelerated the physiological oxidation. Doubling the normal iron content doubled the rate of oxidation, that is, the rate of oxidation was even proportional to the iron content.

Many favorable circumstances occurred which furthered these experiments. Free iron salt has no remarkable catalytic effect, but iron is effective only when it is in complex linkage. Such a complex-forming substance happened to be present in excess in the sea-urchin's egg. But generally it is *not* present in excess. Therefore, generally, with the usual physiological material, the described experiments do not work; the addition of iron to the cell substance has *no* effect on the rate of oxidation.

One can always demonstrate, however, the action of iron on the cell substance by cultivating the cells in solutions containing very small amounts of iron. When the iron in the solutions is used up, the development ceases. If iron is added, the development starts again. I fully realize that these experiments are too ambiguous to prove a relation between iron and enzyme action. Nevertheless, they should come first in all considerations referring to the biological significance of iron.

II. THE HEMIN CHARCOAL MODEL

The consequence of the experiments upon the sea urchins' eggs was that the production of artificial respiration by means of iron was tried, that is, the production of what we call a respiration model. In such a model molecular oxygen must oxidize physiological combustible substances under physiological conditions.

If one brings hemin, the colored component of hemoglobin, to incandescence, a charcoal is formed containing nitrogen and iron. The iron here is linked to the nitrogen. This nitrogen-linked iron acts as a powerful catalyst towards physiological combustible substances. On adding hemin charcoal to aqueous solutions of amino-acids and shaking at body temperature with molecular oxygen, the latter is absorbed and transferred to the amino-acids. Leucine gives valeric aldehyde, ammonia and carbon dioxide. Cysteine gives ammonia, carbon dioxide, sulfuric acid and other unknown products of incomplete combustion. Thus we have here reactions in which, as in the com-

bustion of protein in living cells, molecular oxygen is absorbed and ammonia, carbon dioxide and sulfuric acid are produced.

Narcotics inhibit the respiration of the hemin model, because they displace the amino-acids from the surface of the hemin charcoal. On the other hand, the narcotics are stable on the charcoal surface, so that by removing the amino-acids from the charcoal surface all oxygen absorption is caused to cease. In this way the model reacts exactly as do living cells. The hemin model therefore served to relate narcosis to a simple physical phenomenon: the displacing of reacting substances from surfaces.

The hemin charcoal model has a second property of living cells; traces of hydrocyanic acid render it inactive. As contrasted with the case of narcosis this is brought about by a specific chemical reaction. Hydrocyanic acid combines with the active iron of the hemin charcoal, forming a dissociating chemical component which is catalytically inactive. Traces of hydrocyanic acid are sufficient for this, because the active iron is present only in traces in the hemin charcoal. Thus the hemin charcoal model has served to explain a second fundamental property of living cells, this time chemically.

III. PLATINUM AND IRON CATALYSES

Davy's platinum model was the first respiration-model; the hemin charcoal model was the second respiration-model given us by science. The second model is superior to the first, because it replaces platinum, which never occurs in cells, by a metal which occurs in all cells and which is necessary to life. Common to both models is the heavy metal capable of change of valence. If iron is, as we shall see, the active atom of the respiration enzyme, then we understand the analogy which seemed so remarkable to Berzelius. This analogy was by no means formal; on the contrary, it is deeply founded in the chemical nature of both model and enzyme.

Davy's model satisfied the scientific mood of its time. But the ideas on enzyme action changed, and the hemin charcoal model was contrary to the scientific feeling of its time. Nearly all leading scientists working on enzyme action opposed it and preferred to see in it only formal or incidental similarities with enzyme action. But the younger generation thought that if the phenomena agree, the substances must agree, and concentrated its attention on iron.

Many catalyses of physiological importance were discovered in this way. It was found that cysteine, the only apparently auto-oxidizable cell component, is not really auto-oxidizable, but is oxidized only in the presence of heavy metals. The SH-group of cysteine links iron, copper and manganese to complex components. What seemed to be the auto-oxidation of cysteine is nothing else than the valency change of these complex-linked heavy metals.

Thus a main position of the purely organic enzyme theory was lost. Disappointed it retired into questions of molecular theory; for example, whether the activation of the oxygen or the activation of the hydrogen is more important for the process of respiration. The answer to this question we shall leave to a more advanced time of science. The question of today is the chemical nature of the biological catalysts, while the molecular theory of their action is a question for a later time.

At the same time when biology displaced in its enzyme model platinum in favor of iron, applied catalytic chemistry passed from the platinum to the iron group. The catalytic processes of applied inorganic chemistry, which formerly were platinum catalyses, are now in most cases iron catalyses. Iron is employed, for example, in Germany, to combine atmospheric nitrogen with hydrogen into ammonia by the method of Haber, Bosch and Mittasch. "If we compare the elements in their catalytic value," says Mittasch "keeping in mind the great inorganic industry, the prize will be given to the metals of the iron group, especially to iron itself."

IV. THE INHIBITION OF RESPIRATION CAUSED BY CARBON MONOXIDE

There are three heavy-metal reagents which inhibit the respiration of living cells: hydrocyanic acid, hydrogen sulfide and carbon monoxide. The most important of the three is carbon monoxide. Indeed the experiment which decided in favor of the iron theory was performed with carbon monoxide.

Carbon monoxide reacts as an indifferent gas towards organic substances free from heavy metals. On the other hand, carbon monoxide is bound reversibly by many simple and complex heavy metal salts. The analytical chemist uses copper salt in gas analysis for the absorption of carbon monoxide. The biologist knows carbon monoxide as the gas which displaces oxygen from the iron of hemoglobin.

Eighty years ago Claude Bernard, the discoverer of carbon monoxide hemoglobin, investigated whether carbon monoxide reacts in the body with hemoglobin only or also with the cells. He did not find any action on the cells and thereafter carbon monoxide was considered as the typical blood poison. Later an experiment of John Haldane seemed to confirm this point of view. Haldane added to the air breathed by mice so much carbon monoxide that it displaced all the oxygen from the hemoglobin. The animals died from suffocation. If, however, the oxygen pressure of the respired air was increased from one-fifth of an atmosphere to two atmospheres, the animals did *not* die, though all the oxygen was displaced from the hemoglobin by carbon monoxide. At the higher oxygen pressure the blood *plasma* contained sufficient dissolved oxygen to supply the cells of the body. So it seemed finally proved that only the transport of the oxygen was inhibited by carbon monoxide, but not at all the respiration of the cells.

All those experiments are exact, and yet carbon monoxide does inhibit cell respiration. However, one must not attack the question with the preconceived idea that the same low pressure of carbon monoxide which expels oxygen from hemoglobin is able to inhibit the respiration of the cells. To displace oxygen from hemoglobin the addition of a few tenths per cent of carbon monoxide to the respired air is sufficient. But to inhibit the respiration of the cells a carbon monoxide pressure of the order of magnitude of *one atmosphere* is needed. That is the reason why Claude Bernard, who had become accustomed by his hemoglobin experiments to work with low pressures of carbon monoxide, overlooked the effect of carbon monoxide on cell respiration.

Important for the mechanism of the inhibition of respiration by carbon monoxide is the fact that it depends not only on the carbon monoxide pressure but also on the oxygen pressure. The lower the oxygen pressure, the more pronounced is the inhibition of the respiration at the same carbon monoxide pressure.

The law of distribution of hemoglobin between oxygen and carbon monoxide is well known. It is

$$\frac{HbO_2}{HbCO} \times \frac{CO}{O_2} = K$$

According to the same law the respiration enzyme is distributed between carbon monoxide and oxygen, as was found by respiration measurements at different pressures of carbon monoxide and oxygen. Let FeO_2 and FeCO be the concentrations of the enzyme linked to oxygen and carbon monoxide, respectively. Then the distribution of the enzyme between the two gases is determined by the law

$$\frac{FeO_2}{FeCO} \times \frac{CO}{O_2} = K$$

Because carbon monoxide inhibits respiration anticatalytically, it must combine with the catalyst of respiration. Because this inhibition is reversible, the linkage between the catalyst and carbon monoxide is reversible. These facts prove that the active part of the respiration enzyme is a heavy metal. Carbon monoxide and oxygen compete for this heavy metal as follows from the law of distribution. Just as carbon monoxide displaces the oxygen from the iron of hemoglobin, it displaces the oxygen from the heavy metal of the respiration enzyme. This is the chemically clear mechanism of the effect of carbon monoxide on cell respiration.

V. THE EFFECT OF LIGHT

The great importance of the action of carbon monoxide on cell respiration is due to a strange property of carbon monoxide-iron compounds.

In 1891, Mond and Langer discovered that iron pentacarbonyl splits off carbon monoxide when illuminated. A few years later, in 1897, John Haldane observed that carbon monoxide hemoglobin is split into carbon monoxide and hemoglobin by light. A third case, the photochemical dissociation of carbon monoxide pyridine haemochromogen has been described recently by H. A. Krebs. A fourth case was discovered this year by W. Cremer: the photochemical dissociation of carbon monoxide ferrocysteine, that is, the carbon monoxide compound which is formed when carbon monoxide is passed through a solution of cysteine containing iron. Dewar also worked on the photochemical dissociation of carbon monoxide metal compounds and observed that nickel carbonyl, in contrast to iron car-

bonyl, is not photosensitive. The general experience is that the photochemical dissociation is confined to the *iron* compounds of carbon monoxide. No other heavy metal compound has so far been found which splits off carbon monoxide in the light.

Imagine now a complex iron compound used as a catalyst in some transfer of oxygen. If carbon monoxide combines with the iron, such catalytic reaction will be inhibited by carbon monoxide in the dark.

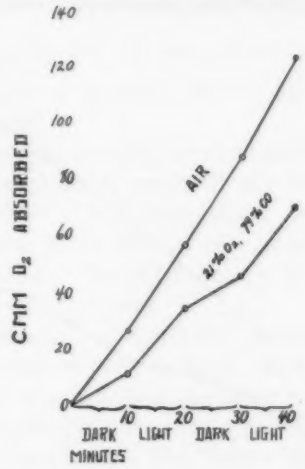

FIG. 1. EFFECT OF CARBON MONOXIDE UPON THE CATALYTIC ACTION OF HEMOPORPHYRIN (DARK AND LIGHT)

But in the light the action of carbon monoxide will disappear because light will set free the catalyst from carbon monoxide.

The experiment has been made with iron porphyrin and iron cysteine as catalysts. Figure 1 represents the porphyrin experiment carried out by Dr. H. A. Krebs. The oxygen transferred is plotted as ordinate, and the time as abscissa. The slope of the curve measures the rate of oxidation. At intervals of 10 minutes the solution was illuminated and darkened, and from the slope of the lower curve one can see that the rate of oxidation increases in the light and falls in the dark. The upper curve represents the rate of oxidation in a carbon-monoxide-free atmosphere, and here we have, as expected, *no* influence of light.

Cell respiration behaves exactly like this simple catalytic system. If we inhibit cell respiration by carbon monoxide and illuminate the inhibited cells, then the inhibition disappears. Figure 2 represents the experiment with living cells. As in the model experiment with iron porphyrin, the absorbed oxygen is plotted as ordinate and the time as abscissa. The slope of the curve measures the respiration. At intervals of 20 minutes the cells were alternately illuminated and darkened, and from the slope of the lower curve one can see that the

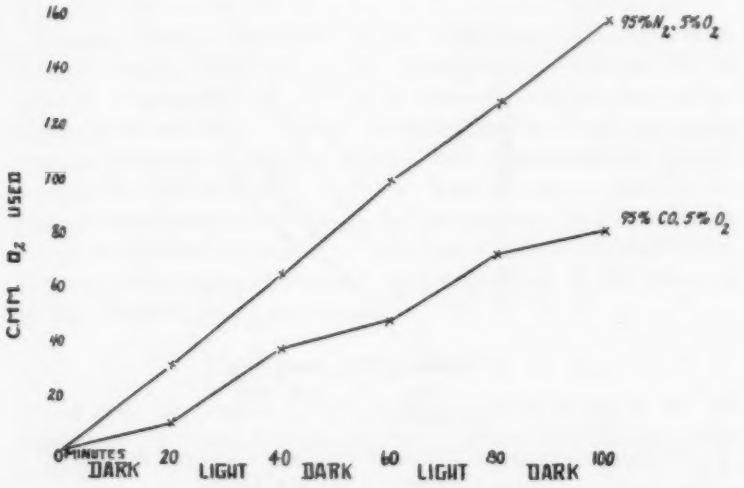

FIG. 2. EFFECT OF CARBON MONOXIDE UPON THE RESPIRATION OF YEAST CELLS (DARK AND LIGHT)

respiration increases in the light and decreases in the dark. The upper curve represents the respiration in a carbon-monoxide-free atmosphere, and here we have no influence of light at all.

As has been said, only the *iron* compounds of carbon monoxide dissociate in the light. Hence it follows from the light experiment with living cells that the heavy metal of the respiration enzyme is *iron*.

The effect of carbon monoxide and light on the cell respiration was discovered in experiments with yeast cells. Later it was found that most cells behave like yeast. I might mention bacteria, plant seeds,

liver, chorion, retina, embryos of chicken and rat, tumors of rat, blood-leucocytes, blood-platelets and so on. All these different cells react in the same way with carbon monoxide. Their respiration enzyme reacts with carbon monoxide reversibly. The enzyme is distributed between carbon monoxide and oxygen according to the law of distribution. The enzyme is sensitive to light when linked to carbon monoxide. That is, in all these cells there is an iron compound which takes up and transfers the oxygen in the process of respiration.

VI. CONCENTRATION OF THE ENZYME IN THE CELL

We now proceed to the question: what is the chemical constitution of this iron compound?

It is well known that complex iron compounds occur in the cell. As F. G. Hopkins discovered, nearly all cells contain cysteine which combines with iron salt to form the complex iron-cysteine. As MacMunn discovered and Keilin recently confirmed, nearly all cells contain iron-porphyrins, *e.g.*, the histohaematin of MacMunn, which is identical with Keilin's cytochrome.

Iron-cysteine *does* combine with carbon monoxide and the carbon monoxide compound is sensitive to light. But the affinities of iron-cysteine to carbon monoxide and the sensitiveness to light are so different from the enzyme that it seems very unlikely that the enzyme is an iron cysteine compound. MacMunn's histohaematin does ***not*** combine with carbon monoxide nor with oxygen, and therefore can not be the enzyme. In addition, histohaematin occurs in such large quantities in the cells that one can see it with the spectroscope—the way in which it was discovered. But the concentration of the enzyme in the cells is almost infinitely small. Indeed one can calculate that 100 million grams of yeast contain only *one* gram of enzyme iron.

From this figure you see that there is not much hope of isolating the enzyme by the methods of analytic chemistry. Moreover, the enzyme is unstable and is quickly destroyed if separated from the cells.

It seems therefore that the old difficulties of the enzyme problem render advance impossible—the instability of the enzyme and its nearly infinitely low concentration. Only a method which is independent of the quantity of the enzyme and which does not require its separation from the cell can overcome these difficulties.

VII. ABSORPTION SPECTRUM OF THE ENZYME:—METHOD

There is such a method. Imagine illuminated cells whose respiration is inhibited by carbon monoxide. Then, as we already know, the respiration increases on account of the dissociation of the carbon monoxide compound of the enzyme. According to the principle of conservation of energy this can take place only if the carbon monoxide compound of the enzyme absorbs the light which dissociates it.

The source of light in the described experiment was a metal filament lamp. The light was a mixture of all the wave-lengths emitted by the metal filament. Now substitute for the metal filament lamp sources of light which emit lines, isolate the lines and illuminate the cells monochromatically. The effect of 15 lines has been examined. These lines were situated in a region which extends from the ultraviolet line, 254$\mu\mu$, to the red line, 660$\mu\mu$. Each of the 15 lines were found to be efficient, each of these lines is therefore absorbed by the enzyme.

But the photochemical efficiency of the different lines was not equal. The efficiency of different lines of equal intensity depended upon the wave-length of the light. The greatest efficiency was found in the blue. The blue line, 436$\mu\mu$, acted 10 times more strongly than the ultraviolet line, 254$\mu\mu$, and 200 times more strongly than the red line, 660$\mu\mu$. The obvious explanation lay in selective absorption.

A general remark on the relation between the *action* of light and the *absorption* of light may be inserted here.

From the light quantum theory of Plank and Einstein, it follows for photochemical dissociation that the number of dissociated molecules must be equal to the number of absorbed light quanta. For gas reactions this simple law has been verified in many cases by Emil Warburg but in aqueous solutions it does not always hold true. For photochemical reactions in aqueous solutions in every new case the relation between the *action* of light and the *absorption* of light must be determined.

This has been done for various carbon-monoxide iron compounds, especially for carbon-monoxide hemochromogen and carbon-monoxide ferrocysteine. Aqueous solutions were irradiated, the absorbed light energy and the carbon monoxide split off were determined. The result was that the photochemical dissociation of carbon-monoxide iron

compounds follows Einstein's law very closely. In the whole region of the spectrum which has been examined one quantum of light decomposes one iron-carbonyl group.

The relation between the *action* of light and the *absorption* of light being thus determined quantitatively, we can calculate by the action of different lines the absorption spectrum of the enzyme. The simple experiment is this: The respiration of cells is inhibited by carbon monoxide in the dark. The cells are then illuminated with different lines of light, whose intensity is measured. From the *action* of light, by means of Einstein's law, the *absorption* of light is calculated.

Here it makes no difference whether the cells contain besides the enzyme other substances which absorb light as long as the total light absorption remains small. Neither MacMunn's histohaematin nor any other accompanying substance, even if it is sensitive to light, disturbs the method. Regardless of what other substances are present, the method gives the absorption spectrum of that substance, the photochemical dissociation of which is measured, that is, in this case, the carbon monoxide compound of the enzyme.

VIII. ABSOLUTE ABSORPTION SPECTRUM OF THE ENZYME:—METHOD

With the described method we obtain the relative absorption spectrum of the enzyme, that is, the positions of the absorption bands and the ratio of the intensities of these bands. But the possibilities of the method are not yet exhausted. It is possible to develop it in such a way as to give also the absolute values of the absorption coefficients. The principle involved is this:

Imagine again cells the respiration of which is inhibited by carbon monoxide in the dark. If we illuminate them, the respiration does not immediately go up. It takes some time, but quite a short period, till the carbon monoxide compound of the enzyme is split by the light. Even without calculation one can understand that the time required to split off the carbon monoxide must be related to the absolute light absorption by the enzyme. If the absorption by the enzyme is strong, then the time required will be short and vice versa.

It is possible to measure this time. We can measure, for example, how long it takes for half of the carbon monoxide compound of the enzyme to be decomposed by a given intensity of light. With this

time and the light intensity used, we can calculate the absolute absorption coefficient for every wave-length. Experimentally, the device is about the same as in the experiment already described. But while in the determination of the relative spectrum the illumination is *continuous*, in the determination of the absolute spectrum *intermittent* illumination is used.

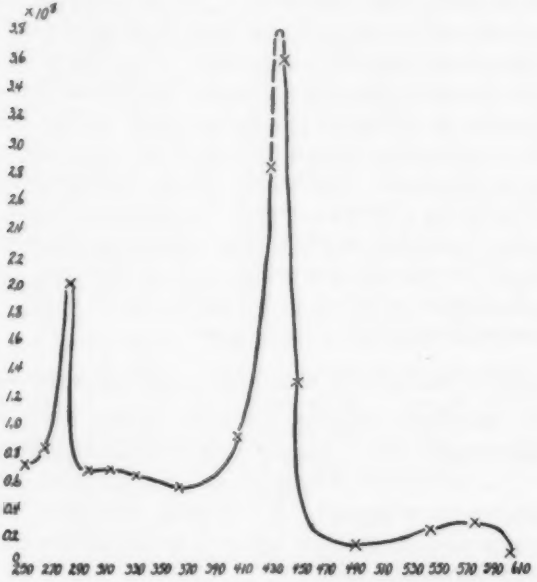

FIG. 3. SPECTRUM OF THE CO COMPOUND OF THE RESPIRATION FERMENT

The absolute absorption coefficient of the enzyme for the blue mercury line 436$\mu\mu$ has been found to be 3.6×10^8 cm^2 per gram atom Fe. This order of magnitude may be illustrated as follows: If *one* gram-molecule of the enzyme be dissolved in one litre, a layer of $\frac{2}{1{,}000{,}000}$ (2×10^{-6}) of a centimetre of this solution will decrease the intensity of light to one half. The light absorption power of the enzyme is,

as you see, very high indeed, of the order of magnitude of our strongest dyestuffs. That it is still impossible to see the enzyme in the cell is due only to its infinitely low concentration.

IX. ABSOLUTE ABSORPTION SPECTRUM OF THE ENZYME

In Figure 3 the absolute absorption spectrum of the carbon monoxide compound of the enzyme is plotted. Fifteen points were determined: Seven wave-lengths in the ultraviolet and eight in the visible part of the spectrum.

To indicate the apparatus used I shall give you for each wave-length the light source out of which it was isolated:

WAVE-LENGTH	COLOR	SOURCE OF LIGHT
$\mu\mu$		
254	Ultraviolet	Mercury lamp
265	Ultraviolet	Mercury lamp
283	Ultraviolet	Magnesium spark
300	Ultraviolet	Mercury lamp
313	Ultraviolet	Mercury lamp
332	Ultraviolet	Zinc spark
366	Ultraviolet	Mercury lamp
405	Violet	Mercury lamp
427	Blue	Carbon dioxide aureole of the tungsten spark
436	Blue	Mercury lamp
448	Sky blue	Magnesium spark
492	Bluish green	Mercury lamp
546	Green	Mercury lamp
578	Yellow	Mercury lamp
603	Orange	Carbon arc containing calcium

We will now compare this spectrum with those of the complex iron compounds of which we know that they occur in all cells. They are iron cysteine and iron porphyrin.

The absolute spectra of these compounds are also measured, not with the indirect method as in the case of the enzyme, but with direct physical methods. The direct absorption measurement with the photoelectric cell of Elster und Geitel is very suitable for this purpose. It is sensitive between 250 and 600$\mu\mu$, which is the range of interest in this case and surpasses in exactness the photographic method.

In Figure 4 the absolute absorption spectrum of the carbon monoxide compound of iron cysteine is plotted. The scale is the same as in the figure of the enzyme spectrum. The positions of the bands as well as the absolute values of the absorption coefficients show that iron cysteine and enzyme are different substances.

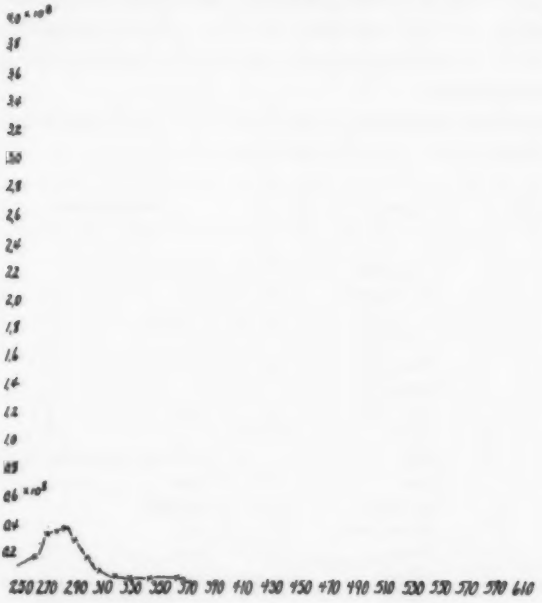

FIG. 4. THE ABSORPTION SPECTRUM OF THE CO COMPOUND OF IRON CYSTEINE

In Figure 5 the absolute absorption spectrum of carbon monoxide hemin is plotted. The scale is again the same as in the figure of the enzyme spectrum. The concordance of this spectrum with that of the enzyme (Fig. 3) is remarkable, both in the positions of the bands and in the absolute values of the absorption coefficients. The closeness of this agreement may be shown with a few figures.

A minimum of absorption lies in both cases around 490$\mu\mu$. The absolute absorption coefficients here are:

For carbon monoxide hemin..... $0.12 \times 10^8 \frac{\text{cm.}^2}{\text{gram atom Fe}}$

For carbon monoxide enzyme..... $0.13 \times 10^8 \frac{\text{cm.}^2}{\text{gram atom Fe}}$

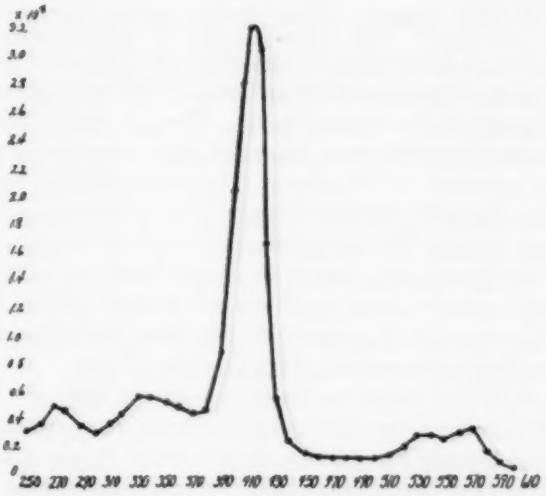

FIG. 5. THE ABSORPTION SPECTRUM OF CARBON MONOXIDE HEMIN

The maximum of absorption lies in both cases in the blue. The absolute absorption coefficients in the maximum are:

For carbon monoxide hemin..... $3.2 \times 10^9 \frac{\text{cm.}^2}{\text{gram atom Fe}}$

For carbon monoxide enzyme..... $3.6 \times 10^9 \frac{\text{cm.}^2}{\text{gram atom Fe}}$

From this quantitative agreement it follows that the enzyme is an iron-porphyrin compound, which was expected from the experiments with iron-porphyrin as catalyst. But now it is proved.

If at this point we look back, we see that in many respects the hemin

charcoal has been more than a model. It is true that in the hemin charcoal the porphyrin is destroyed by the heat of incandescence, but still there is the nitrogen of the porphyrin and the linkage of the iron to the nitrogen. Iron linked to nitrogen is the catalyst in both, in the artificial respiration of the hemin charcoal model and the respiration of living cells.

X. THE CHEMICAL CONSTITUTION OF THE ENZYME

Porphyrin is a collective name for compounds built up of four pyrrol nucleii, the constitution of which has been elucidated by the analytical and synthetical work of Hans Fischer. The side chains of the pyrrol nuclei in the porphyrins may be varied; these may be, for example, methyl, ethyl, vinyl, or acid groups. Thus we get different porphyrins. All of these different porphyrins react with iron, yielding complex iron compounds in which the iron is linked to the pyrrol nitrogen. All the different iron porphyrins react with organic bases and with proteins, as has been recently shown by Anson and Mirsky. There is quite a world of iron porphyrin compounds possessing different side chains on the porphyrin nucleus and linked to various bases.

None of the iron porphyrin compounds known until now is, as the absorption spectra show, identical with the enzyme, neither is hemin, nor hemoglobin, nor MacMunn's histohematin. In fact none of these iron porphyrins possess the catalytic property of the enzyme. Hemoglobin does combine with oxygen, but is not capable of activating it. Histohematin can transfer activated oxygen, as was shown by MacMunn in 1886, but the most important property of the enzyme is missing: the property of reacting with molecular oxygen. Hemin does react with molecular oxygen and is capable of transferring it to some organic substances. But its catalytic value is not comparable with the enzyme in magnitude.

Thus we see the problem is still to be solved. Although we know the class of substances to which the enzyme belongs, its exact chemical constitution is yet unknown. The task is now to find the iron porphyrin not only similar to the enzyme but identical with it.

XI. THE RESPIRATION ENZYME AND OXIDASES

At the end a word of defense. I have used in this lecture the term "respiration-enzyme" which seems to be in contradiction to the multitude of oxidases which have been found in cell extracts.

If living cells are crushed and the pulp is extracted under certain favorable conditions, then the entire respiration passes into the extract.

The respiration in the extracts is unstable. Usually the decay begins with a decrease of the carbon dioxide development. Soon the oxygen absorption decreases so that at the end the extract solutions may oxidize some sensitive organic substances like phenols or polyamins a little more quickly than water. But the essential effects of respiration have disappeared.

If one calls enzymes that transfer molecular oxygen oxidases, the extracts contain oxidases. If one classifies the oxidases according to their effect, one has in the extracts at different times different oxidases: glucose oxidase, alcohol oxidase, indophenol oxidase and so on. Strictly speaking there exist as may different oxidases as extraction experiments. And if the oxidases were preformed in the cells, innumerable oxidases would exist.

The investigation of living cells has not confirmed this. If a fraction of the respiration is inhibited by carbon monoxide, the uninhibited respiration is in every respect the same as the inhibited fraction. Light absorption coefficients especially are equal for every fraction of respiration. But if one part of the oxygen were transferred by iron cysteine and another part by iron porphyrin, different spectra would be found corresponding to different fractions of respiration. This type of analysis can be carried until 99 per cent of the respiration is inhibited by carbon monoxide. It follows from this that all iron atoms which transfer the oxygen in respiration, in one kind of cell, are linked identically.

Therefore if different oxidases occur in an extract of one kind of cell they are not enzymes which were preformed in the living cell, but transformation and decomposition products of a substance uniform in life. Uniformity of respiration enzyme and multitude of oxidases in the extracts do not constitute a contradiction.

Another question is whether the respiration enzyme, uniform in one type of cell, is different in different types of cells. Essential differences have not been found so far, not even by comparing cells as different as yeast and the cells of the retina. But it may be that there are cases in nature in which iron is replaced by another heavy metal or the porphyrin by another complex forming substance.

THE CIRCULATORY FAILURE OF DIPHTHERIA

III. The Treatment of the Circulatory Failure of Diphtheria

FRANCIS F. SCHWENTKER AND WILLIAM W. NOEL

From the Sydenham Hospital for Infectious Diseases and the Department of Pharmacology, Johns Hopkins University, Baltimore, Maryland

In a previous communication (1), we have discussed the classification and mechanism of the circulatory failure of diphtheria. Evidence was presented supporting our views that the circulatory abnormalities of this disease may be divided into two groups, purely on the basis of the clinical picture presented. The first of these is the early type, occurring as an essential part of the general toxemia; the second is the late type, in which symptoms appear usually quite suddenly during apparent convalescence. The mechanism of failure in these two types is entirely different; circulatory collapse in the early cases is caused by a direct action of the diphtheria toxin on the tissues of the vascular system. The cause of the late failure, however, is more obscure but is probably due to a series of local inflammatory reactions set up in the heart muscle and the conducting system during the process of regeneration and repair.

With this in mind, therefore, we have undertaken a further study of the circulatory failure of diphtheria in an attempt to understand better the mechanism underlying each type and to evolve therefrom a logical basis for treatment. Three hundred and eighteen consecutive cases of diphtheria were studied at the Sydenham Hospital during 1928 and 1929. Of these, 28 showed marked evidences of circulatory failure; 19 were classed as the early type, while 9 fell into the group of late circulatory deficiency. Further observations from an experimental standpoint were made on rabbits under the influence of diphtheria toxin. And finally, studies were made of the case records of 1600 diphtheria patients admitted to the hospital from 1920 to 1927.

The results of these studies have been presented in a previous paper

(2). Briefly, it was shown that in diphtheria intoxication there is a profound disturbance of the carbohydrate metabolism. This is manifested in the early stages by an increased glycogenolysis and carbohydrate utilization, so that in severe cases a marked hypoglycemia is evident. The liver and muscles lose their entire glycogen supply. If the toxemia be of longer duration there is an increasing difficulty in the assimilation of dextrose from the blood stream and a progressive hyperglycemia results. This is probably due to a suppression of the endogenous products of the pancreas since insulin, given by injection, causes the assimilation of the excess dextrose. The pathological effects of this increased utilization and demand for carbohydrates are added to the decreased ability of the body to assimilate the dextrose available and nutritional disturbances are set up throughout the body. Such disturbances usually become evident first in deficiencies in the circulatory mechanism, since this set of tissues can never be placed entirely at rest.

A consideration of these facts suggests a method of treatment. If carbohydrate were supplied to the body through intravenous injections of dextrose and then made available by the administration of insulin, these nutritional disturbances might be balanced. In this way, the toxic patient showing signs of circulatory collapse might receive sufficient support to allow the antitoxin to counteract the toxemia and ward off impending death.

We have treated 14 patients suffering from diphtheria with intravenous injections of dextrose. All of them were so toxic that death was imminent; several were unconscious. Three of the patients showed no symptoms referable to circulatory embarrassment; four showed a loud systolic murmur. In one case there was an enlarged, painful liver but no other sign of a failing circulation. Three patients presented a systolic murmur and an engorged liver only; in two cases there was an evident cardiac arrhythmia in addition to the murmur and enlarged liver, while in one patient a systolic murmur, an enlarged liver, an arrhythmic pulse and protodiastolic gallop rhythm gave evidence of a severely damaged circulatory system.

In 13 of these cases the patients recovered. In most instances the symptoms were rapidly alleviated; the temperature and pulse rate

returned to normal within 24 hours after the injection, the toxic condition disappeared, and convalescence was uneventful. Two of the patients, however, remained toxic for five days despite large doses of antitoxin and frequent intravenous injections of dextrose. From the throat of these patients hemolytic streptococci were cultured in addition to the Klebs-Loeffler bacilli. In one case death occurred four hours after admission. This patient, however, had been ill for days and was moribund when first seen.

Following are the protocols of the most characteristic cases treated. Summaries of the remainder have been omitted for the sake of brevity.[1]

Case 10583, colored female, age 7 years, admitted on the 10th day of illness with a diagnosis of nasal and tonsillar diphtheria. She was very toxic, almost unconscious and showed on examination a profuse, bloody, membranous nasal discharge and an extensive diphtheritic membrane on the tonsils. Temperature 101°F., pulse rate 125, markedly irregular. B.P. 115/65. A loud systolic murmur, showers of extrasystoles and a definite protodiastolic gallop rhythm were heard on auscultation over the precordium. The liver was palpable 3 cm. below the costal margin.

Forty thousand units of antitoxin were injected intramuscularly. 200 cc. of a 10 per cent dextrose solution, 7 units of insulin and 10,000 units of additional antitoxin were given intravenously. Eight hours later, the temperature, which had risen to 103.6° after injection of the antitoxin was 98.6°F.; the pulse rate had fallen from 125 to 85 per minute and the patient was fully conscious. Extrasystoles were still heard but were fewer in number; the gallop rhythm was no longer present. By the following day, the 12th day of illness, the extrasystoles had also disappeared and the liver edge was no longer felt. On the 13th day the systolic murmur could not be heard and the patient was obviously improved. From then on, the convalescence was uneventful and the patient was discharged from the hospital on the 49th day.

Case 10863, white female, age 7 years, admitted on the fifth day of illness with a diagnosis of tonsillar, pharyngeal and nasal diphtheria. She lay in a semi-stuporous state from which she could be aroused only with difficulty; she was exceptionally toxic. There was a profuse, bloody, membranous nasal discharge and the tonsils were covered with diphtheritic exudate. Temperature 101°F.,

[1] Following are the history numbers of those cases for which protocols have been omitted. Summaries may be obtained from the historian: 10881, 10983, 11154, 11189, 11237, 11284, 11301, 11372, 11430, 11433.

pulse 125, B.P. 115/80. The heart sounds were loud and gave the impression of a labored myocardium, but there was no arrhythmia or murmur. The liver was palpable 4 cm. below the costal margin.

She was given 20,000 units of diphtheria antitoxin intramuscularly and 20,000 additional units intravenously. 10 grams of dextrose were then injected intravenously, but insulin was omitted since it was desired to study the carbohydrate tolerance. The following day, the sixth day of illness, she was somewhat improved but the pulse rate had risen to 135 per minute and she was quite cyanotic. Twenty cubic centimeter of 50 per cent dextrose were administered intravenously and supplemented by 6 units of insulin intramuscularly; a sub-pectoral infusion of normal saline was begun. On the seventh day of illness she was quite conscious; the liver was no longer palpable and the cyanosis had disappeared. Pulse 130. Two hundred cubic centimeters of 10 per cent dextrose were given intravenously with 6 units of insulin intramuscularly. Following this, the temperature and pulse rate began a rapid descent to normal. During convalescence she developed the pulse irregularity common in the late type of circulatory failure but this was evident only a few days. An electrocardiogram made on the 40th day showed a normal mechanism. Her condition was excellent and she was discharged on the 58th day.

Case 10803, white female, age 4 years, admitted on the fifth day of illness with a diagnosis of nasal, tonsillar and pharyngeal diphtheria. She was very toxic, temperature 101.2°F., pulse 135, B.P. 112/60. The tonsils and pharynx were covered with membrane and the nose exuded a bloody, membranous discharge. There was a bloody diarrhea. The heart showed a loud systolic murmur and showers of extrasystoles, with marked variation in the force of the beats. The second sound over the pulmonic area was split.

Thirty-three thousand units of antitoxin had already been given intramuscularly some hours previously, so that 10 grams of dextrose in 50 per cent solution and 5 units of insulin were given intravenously. Four hours later the pulse irregularity was no longer present and by the following morning only a faint systolic murmur was audible at the apex. The general toxemia improved rapidly. On the 36th day she showed, at times, a few extrasystoles but these were heard for only three days, and on the 50th day an electrocardiogram showed a normal mechanism. She was discharged in excellent condition on the 51st day. It is interesting that during the first three days of her hospitalization, acetone bodies were found in her urine, indicating the extreme need for dextrose. This was common in many of the patients and disappeared rapidly under dextrose therapy.

It is difficult to depict all the minor points by which one judges the condition of a patient, so that in a description such as this is, it becomes necessary to resort chiefly to a mere statement of the case.

However, we feel that these brief protocols give evidence that each of the patients showed rapid improvement, following the administration of the dextrose, from a very grave condition. We believe, that, in most of these cases, death would have supervened had the patients not received sufficient dextrose to supply nutrient material to a rapidly failing circulatory system. For this reason, we have adopted in cases of severe toxemia and early circulatory failure in diphtheria, the following general management: The patient receives immediately on admission a large dose of antitoxin intramuscularly. One-half hour later, if no reaction has resulted, 10,000 to 20,000 units of additional antitoxin and 20 grams of a 50 per cent solution of dextrose are injected intravenously. Ten to twenty units of insulin are supplied intramuscularly. We prefer, in this stage, to supply the fluid by the subcutaneous or intraperitoneal route rather than risk an added load on a failing circulation. Should the patient be restless, which is rarely the case, sedatives are given to obtain complete rest. At intervals varying from 12 to 24 hours, according to the degree of toxemia, the intravenous injection of dextrose is repeated, each time supplemented by insulin. The quantity is so chosen that 1 unit of insulin is given for each 1 to 2 grams of dextrose. The more toxic the case, the greater the amount of insulin necessary to cause complete utilization of the carbohydrate. After the first or second injection, the dextrose may be given as a 10 per cent solution in order to supply the needed fluid. The administration of dextrose and insulin in this manner is repeated until the patient's condition is satisfactory and recovery seems certain.

There is no rationale in treating cases of late circulatory failure in this manner, since the patient is already in convalescence and the carbohydrate metabolism has returned to normal. It seems to us, however, that the administration of dextrose in the early stages would lessen the degenerative processes in the myocardium and thus decrease the late inflammatory reaction which is so apt to cause delayed circulatory failure. It may be significant that, despite the toxic condition of all of the cases treated with dextrose, only two developed any sort of late circulatory abnormality and these were only transient extrasystoles.

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NOTE ON THE EFFECT OF REPEATED INTRAVASCULAR INJECTIONS OF HEPARIN

W. H. HOWELL AND C. H. McDONALD

From the School of Hygiene and Public Health, Baltimore, Md.

In a previous communication (1) one of us has referred to the fact that when purified heparin is injected intravenously into a dog in quantities sufficient to render the entire volume of blood incoagulable the effect wears off rapidly. Blood withdrawn one hour after the injection is no longer incoagulable, although the time of coagulation is greatly prolonged. Two hours after the injection clotting takes place in 30 to 40 minutes, as compared with a normal time of 8 to 10 minutes, while after three hours the coagulation time is practically back to normal. Single injections of the purified heparin in man or in the dog seem to be entirely without injurious effects, and it has been suggested that the heparin might be used to advantage in cases of venous thrombosis in man. Owing, however, to the transitory effect of the heparin when given *in vivo* it would seem to be necessary, if so used, to repeat the injections at certain intervals. In this connection the question arose whether repeated injections would provoke some deleterious reaction in the blood itself. It seemed possible that the blood might exhibit some kind of over-compensation which would result in a shortening of the coagulation time. The following experiment was made to throw light upon this point.

A young dog was used, weighing 13.5 pounds, and for five successive days a specimen of blood was taken from the external jugular vein by vene-puncture to determine the normal coagulation time, the number of red and white corpuscles and blood-platelets and the range of variation in these factors. Then for six successive days heparin was injected intravenously in an amount sufficient to render the blood incoagulable, assuming the blood to be equal to $\frac{1}{16}$ the weight of the animal, and blood-counts and tests of coagulation time were made twenty-four hours after each injection.

The procedure used in making the observations was briefly as

follows. The animal was trained to lie quietly upon the table while a hypodermic needle was inserted into the vein through the skin. Four cubic centimeters of blood were taken. Two cubic centimeters were used to make duplicate tests of the coagulation time, 1 cc. in each tube. From one of these tubes enough blood was removed with a counting pipette to make a determination of the number of white cells. Of the remaining blood 1 cc. was mixed with 9 cc. of a Rees and Ecker solution, and 1 cc. with 9 cc. of a Leake and Guy solution. These mixtures were made in 10 cc. specific gravity flasks which, when stoppered, were entirely free from air and could be shaken throughly. Two glass beads were introduced into each flask to facilitate mixing. Specimens from these flasks were further diluted, each with its own solution, in a 1 to 20 pipette so as to give a final dilution of 1 to 200 for the counting of the red cells and the platelets. Immediately after the removal of the 4 cc. of blood the barrel of the syringe was detached from the needle which was left in the vein, and with a new syringe 2 cc. of a 0.9 per cent sterilized solution of sodium chloride were injected into the circulation in the preliminary observations, and 2 cc. of a sterilized solution of heparin (13.5 mgm.) in the heparin observations.

The coagulation time was determined by emptying 1 cc. of blood from the syringe into a clean vial of 15 mm. internal diameter and noting the time before it became solid. It is well known that very slight variations in conditions influence markedly the time of clotting. In some cases the operator was not successful in penetrating the vein at the first insertion of the needle and in such cases admixture of more or less tissue-juice made a possible disturbing factor in the regularity of the clotting time.

In counting the blood-platelets the methods and solutions recommended by Rees and Ecker (2) and by Leake and Guy (3) were employed. The solution proposed by Leake and Guy was the more satisfactory in some respects, but it had the disadvantage that in some cases it caused clumping of the erythrocytes which made a count of either the red cells or the platelets impossible. This effect was not observed with the blood of other animals (man, rat, guinea-pig, rabbit, cat) nor did it always occur with dog's blood. Some tentative observations indicated that the clumping with dog's blood was attributable to the high percentage (6 per cent) of formaldehyde, and that this

effect fell out when the percentage of this ingredient was reduced (2 per cent).

The results of the experiment are given in Table I. They indicate that a daily injection of a relatively large dose of purified heparin for six consecutive days does not cause any change in the corpuscles of the blood, nor any significant change in the clotting time. There was certainly no indication of a shortening of this time, but rather a slight tendency toward a lengthening.

TABLE I

DATE	RED CORPUSCLES		PLATELETS		WHITE CORPUSCLES	CLOTTING TIME	REMARKS
	R. and E.	L. and G.	R. and E.	L. and G.			
Preliminary observations							
						minutes	
Nov. 7	5,950,000	6,212,000	350,000	320,000	17,000	7.5	
Nov. 8	5,000,000	Clumping	280,000		17,400	7.5	
Nov. 9	5,960,000	6,190,000	510,000		14,720	7.5	
Nov. 11	5,900,000	Clumping	350,000		14,880	7.0	
Nov. 12	6,020,000	Clumping	380,000		15,680	8.5	
Nov. 14	6,300,000	6,580,000	340,000	320,000	11,200	12.0	Full dose of heparin injected
Heparin observations							
Nov. 15	5,780,000	Clumping	340,000		11,000	Lost	Heparin injected
Nov. 16	6,060,000	5,910,000	340,000	290,000	12,000	17	Heparin injected
Nov. 17	6,010,000	Clumping	280,000		12,960	10	Heparin injected
Nov. 18	6,380,000	Clumping	340,000		17,340	8	Heparin injected
Nov. 19	6,280,000	Clumping	330,000		14,560	13	Heparin injected
Nov. 20						15	Heparin injected

The animal remained in the best of condition throughout the experiment. There was not the slightest indication of any deleterious effects following upon the injections.

After the injections of heparin were begun the daily urine of the animal was collected and evaporated to dryness upon the water-bath. The dried residue was treated with water, filtered, and the filtrate was then precipitated by the addition of an equal volume of acetone. The precipitate was centrifugalized off and again treated with

water. This solution after filtration was again precipitated with acetone and the process was repeated several times. The material finally obtained was tested for its anticoagulant action upon blood. It was found that 1 mgm. sufficed to prevent completely the coagulation of 1 cc. of blood. There can be little doubt, therefore, that the excess of heparin injected into the blood was removed, in part at least, through the kidneys.

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RECURRENT AGRANULOCYTOSIS[1]

B. H. RUTLEDGE, O. C. HANSEN-PRÜSS AND W. S. THAYER

From the Department of Medicine, the Johns Hopkins University

Within the last several years the literature on agranulocytic angina and on agranulocytosis in general has become voluminous. The clinical picture is familiar. The subject has been discussed at length in reviews such as those of

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Aubertin, C. and R. Lévy: L'agranulocytose et les syndromes agranulocytaires. *Arch. d. mal. du coeur*, 1928, xxi, 369–473.

[1] Submitted for publication November 2, 1929.

It has been our good fortune to observe a patient who presents a clinical picture, unique in medical literature, so far as we are aware; namely, a cyclic agranulocytic angina with leukopenia but without anaemia, which is apparently of nearly twenty years' duration.

The patient, who is now 19 years of age, has been studied by good observers from time to time, since early infancy when he had already begun to show the peculiar symptoms which he now manifests. The constancy of these symptoms and the regularity of their recurrence are such as to suggest to us that the picture cannot be unique; and although our opportunity to study these manifestations has not been what we could have wished, it seems to us worth while to place our observations on record.

The patient (X), 19 years of age, is the son of well-to-do parents. His mother is 50 years of age and in good health, except for moderate hypertension. She has had four pregnancies, all in the fourth decade. Three of these ended in miscarriages. The patient is the result of the second pregnancy. The father, 69 years old, is markedly arteriosclerotic and has intermittent claudication. There is no history of disease of the blood in the immediate or collateral families. Bordet-Wassermann tests of the blood of both parents have been repeatedly negative.

Childhood. At the time of the patient's birth, the mother was 31, the father 50 years old. The birth, premature, at 8 months, was induced instrumentally because of eclampsia. The weight at birth was $5\frac{3}{4}$ pounds. The child appeared otherwise normal. He did not, however, gain weight satisfactorily, and at the age of 8 weeks, had an attack of suppression of urine which yielded following hot packs over the kidneys. On 7, Sept., 1908, one month later he passed no urine for a period of 36 hours. The first few drops of urine thereafter contained blood. One week later he had a similar attack.

Feeding was always a difficult problem. The child did not thrive, and suffered frequently with "spell after spell of indigestion and diarrhoea" associated with the passage of greenish, lumpy stools.

When two and a half months old, he suffered from extensive furunculosis for which he was treated by Dr. Medwin Leale of New York City. While under this physician's care, he was observed through recurrent attacks of furunculosis, accompanied by irregular, remittent

fever lasting from three to ten days. Occasionally, during these attacks, an aphthous patch appeared in the buccal mucosa followed by considerable necrosis. On two or three occasions, there were concomitant gastro-intestinal disturbances but never any indications of an ulcerative colitis. Repeated blood examinations carried out during these attacks disclosed a leukopenia with a well marked relative lymphocytosis. The number of red blood corpuscles and the percentage of haemoglobin were within normal limits. A representative differential blood count was as follows:

	per cent
Polymorphonuclear neutrophiles	1
Polymorphonuclear eosinophiles	0.8
Polymorphonuclear basophiles	0.2
Large lymphocytes	32.2
Small lymphocytes	65.8

From the pus of the furuncles *Staphylococcus albus* was cultivated repeatedly. The use of an autogenous vaccine was followed by almost complete cessation of the furunculosis.

Dr. Leale recognized the association of the furunculosis with the abnormal blood picture and published a report of the case. (Leale, Medwin: Recurrent furunculosis in an infant showing an unusual blood picture. *J. Am. Med. Ass.*, Chicago, 1910, liv, 1854.)

The baby did fairly well except for his periodical "attacks," until the age of 13 months, during the summer of 1909, when great difficulty was experienced in feeding him, and he again began to suffer with attacks of diarrhoea. Dr. L. Emmett Holt was consulted and recognized the periodicity of the above mentioned attacks.

Boyhood. These periodical "attacks" continued to recur usually at intervals of three weeks. A typical attack was described approximately as follows: He looks yellowish. There is a little fever—99° to 100°F. (rectal); he feels listless and fatigued. The temperature gradually rises to 104° or 105° reaching its height on the fourth day, when it gradually falls; it is irregular, suggesting sepsis. The gums become red and swollen, and show more or less numerous, usually rather deep, indurated sores which appear also on lips, tongue and buccal surfaces from which Vincent's organisms have been found on several occasions in abundance. The glands of the neck swell.

With the fall of temperature the buccal manifestations disappear. The patient may sleep through most of the attack. At other times he is very irritable and fretful. Occasionally he vomits. There is anorexia. The bowels remain regular. It has been observed from time to time, that the white blood cell count is low during the attack, with a marked depression, chiefly of the granular elements. During the summer of 1916, he had three "attacks" without the occurrence of a sore mouth.

The patient has had none of the contagious diseases of childhood. In 1916 he was ill with "pneumonia" for three weeks. During this illness he suffered with one of his usual "attacks," associated with a pronounced leukopenia. Immediately after the "attack" the leucocytes increased in number, and he recovered from his pneumonia.

In December, 1918, he was ill with influenza and pneumonia. During this illness the leukocytes on one occasion were recorded as 800 per cubic millimeter of blood.

In April, 1919, he was admitted to the Harriet Lane Home of the Johns Hopkins Hospital for two week's observation. The physical examination at this time showed little except large, infected tonsils, and a slight retraction of the right ear drum. While in the hospital, he was studied during an "attack:"

This began by a gradually rising temperature which reached its fastigium on the fourth day (103.2°, rectal), and gradually fell, reaching a normal point six days later. Examinations of the blood revealed the following picture:

15, April, 1919. First day of the "attack." Red blood corpuscles, 5,160,000 to the cubic millimeter. Haemoglobin, 82 per cent (Sahli). Colourless corpuscles, 4,600 to the cubic millimeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	25
Polymorphonuclear eosinophiles	7
Polymorphonuclear basophiles	1
Small lymphocytes	46
Large mononuclears	16
Transitional forms	4
Myelocytes	1

21, April, 1919. Colourless corpuscles 4,720 to the cubic millimeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	28
Polymorphonuclear eosinophiles	3
Small lymphocytes	52
Large mononuclears	14
Transitional forms	3

At this time he had an otitis media from which pus containing *Staphylococcus albus* was obtained.

In May, 1919, he returned to the Harriet Lane Home for a tonsillectomy and adenoidectomy. Again he was followed through another similar "attack" with the following blood picture.

29, May, 1919. Colourless corpuscles 4,520 per cubic millimeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	8.5
Polymorphonuclear eosinophiles	4
Polymorphonuclear basophiles	5.5
Small lymphocytes	63.5
Large mononuclears	10
Transitional forms	8.5

Two days later the leukocytes were 5,500 to the cubic millimeter, and two days after this, 6,200. The elevation of temperature, which was not high at any time, lasted for eight days. After his discharge from the hospital, he remained in essentially the same condition and continued to have the periodical recurrences of fever and disability. In 1920, he went through a third siege of "pneumonia," but unfortunately there are no records of a blood examination during this illness.

Present condition. Owing to the severity of the recurrent seizures and the accompanying disability, the patient has been unable to attend school with others of his age, and though not slow mentally, he is backward in his studies. At home he has been humoured in such manner that he has become very wilful and unrestrained. He is given to long and futile discussions with his mother and when feeling ill, at the time of his attacks, he gives way to paroxysms of anger in which he is actually violent in his behaviour toward his mother and

others about him. There can be little doubt that, in a certain sense, these paroxysms are purposeful in that he is consciously or subconsciously aware that those about him are cowed, and that he gains his ends. On the other hand there seems to be no evidence of definite mental defect. This was supported by an examination by Dr. J. R. Oliver.

On 11, April, 1928, he came under the observation of one of us (Thayer). He was a healthy looking young man, white, aged 19. Height, 5 feet 6½ inches (in shoes). Temperature, 97.7°F. (mouth); vital capacity 3,150 (calculated normal vital capacity 3,600). Blood pressure 114/65. He was well built. The hair on the face was not developed to the usual extent. Pubic hair rather transversly arranged. His voice was "changing" like that of a boy at puberty. In other respects, however, he showed no outward physical abnormalities. The genitalia were normally developed. The colour was good. The pulse, 76 per minute. There was nothing unusual in eyes, ears, nose or throat (Dr. Crowe). There was a pronounced gingivitis; the gums were much swollen and bled very readily; there was a well marked pyorrhea alveolaris. Roentgenograms showed several areas of periapical disease and moderate absorption of the alveolar crests with chronic pericementitis. Dr. Coriell reported a typical Vincent's infection. This was confirmed bacteriologically. Several cervical lymph glands were palpable; both epitrochlears, just felt. The heart and lungs showed no abnormalities. Abdominal examination revealed nothing unusual. The spleen was not felt. The reflexes were normal. Eye-grounds, normal.

Blood examination: Red blood corpuscles, 5,200,000 per cubic millimeter. Haemoglobin 90 per cent (Dare). Colourless corpuscles, 6,900 per cubic millimeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	36
Polymorphonuclear eosinophiles	9
Polymorphonuclear basophiles	2
Small lymphocytes	28
Large lymphocytes	3
Large mononuclear and transitional forms	11
"Smudges"	11

Fluoroscopic examination of the chest and Roentgenograms of the head showed no abnormalities. X-ray studies showed a rather spastic gastro-intestinal tract with appearances somewhat suggestive of adhesions in the right lower quadrant.

Cultures of blood obtained 24, April, 1928, showed no growth after 9 days in broth, glucose and plain agar plates.

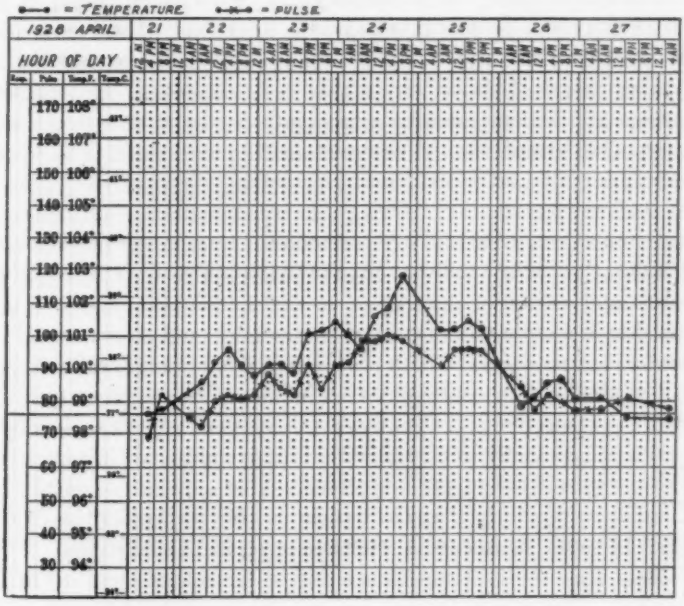

CHART I

Urine: Slightly turbid, amber. Acid. Specific gravity, 1.030. Sugar—negative. Albumin—negative. Microscopic: Many triple phosphates. No pus cells, casts or red blood corpuscles.

'Phthalein excretion: 1 hour, output 50 per cent; 2 hour, output 10 per cent; Total 2 hours 60 per cent.

The non-protein nitrogen in the blood was 31 mgm. per cent; uric acid 4.6; sugar 88. Carbon dioxide Combining power, 551 volumes per cent. Bordet-Wassermann—negative.

Gastric analysis: 12 cc., only, obtained in 30 minutes. Much ropy,

clear mucus. Contents well chymified. No gross blood. Total acidity, 69 ac. per cent. Free HCl, 59 ac. per cent. Combined acidity, 10 ac. per cent. No lactic acid or occult blood.

Repeated stool examinations showed no abnormalities.

While under observation he was studied during four distinct "attacks" which occurred roughly at three weeks' intervals, the first only at the hospital. The patient has grown so accustomed to the periodicity of his disease that he is able to predict, almost to a day, the recurrence of a new "attack." The manifestations were similar to those described above, except that at no time was there jaundice. Fatigue and listlessness with a moderate degree of drowsiness were outstanding symptoms. A severe stomatitis with swelling and soreness of the gums and buccal mucous membranes, aggravated by numerous shallow ulcerations of varying size, was observed on all occasions. Sometimes the ulcerations were surrounded by a rather marked zone of induration; this condition often interfered with the opening of his mouth and motion of the tongue, so that he avoided eating. There was always a more or less evident enlargement and tenderness of the cervical lymph glands. With the onset of these changes the temperature gradually rose, reaching its fastigium on the fourth or fifth day when the subjective and objective manifestations were at their height; it then fell gradually to normal within six to eight days, in association with the subsidence of the symptoms. This sequence of events has occurred so regularly that the patient is able to predict the day on which he would be free from fever and unpleasant symptoms.

Chart I shows the temperature during the attack observed in the hospital. This is said to have been an unusually mild attack. The blood was examined daily before, during and after an "attack." In an interval between attacks the blood picture was as follows:

7, May, 1928. Red blood corpuscles, 5,010,000 per cubic millimeter. Haemoglobin, 97 per cent (Dare). Colourless corpuscles, 6,475 per cubic millimeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	56
Polymorphonuclear eosinophiles	10
Polymorphonuclear basophiles	3.5
Small lymphocytes	12.5

Large lymphocytes	2.5
Large mononuclear transitional forms	7
"Smudges"	8.5

The platelets were numerous, large and coarsely granular. The bleeding and clotting time were normal. Shortly before an attack there was usually a sudden drop in the number of colourless corpuscles with a change in the relative proportions of the various elements.

11, May, 1928. Red blood corpuscles, 5,850,000 per cubic millimeter. Haemoglobin, 95 per cent (Dare). Colourless corpuscles, 2,450 per cubic millimeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	16
Polymorphonuclear eosinophiles	11
Polymorphonuclear basophiles	4
"Mast cells"	2
Small lymphocytes	29
Large lymphocytes	11
Abnormal lymphocytes	8
Lymphoblasts	1
Large mononuclear and transitional forms	8
Myelocytes	6
Plasma cells	3
Megacaryocytes	1

At this time the patient was rather listless but free from subjective symptoms. The temperature was normal. On the next day he was more listless and his appetite was poor. The temperature remained normal. On 14, May, he complained of general aching and a sore and tender mouth, tongue and gums. The temperature was still normal

On 15, May, four days after the first blood examination, red blood corpuscles, 5,750,000 per cubic millimeter. Haemoglobin, 90 per cent, (Dare). The colourless corpuscles were 4,670 per cubic millimeter. The differential formula was as follows:

	per cent
Polymorphonuclear neutrophiles	0
Polymorphonuclear eosinophiles	11.5
Polymorphonuclear basophiles	1.5
Small lymphocytes	28
Large mononuclear and transitional forms	36
Myelocytes	2
"Smudges"	9

The patient felt very badly, weak and "generally knocked out." The temperature was 99°F. (mouth); the pulse rate, not elevated. The mouth was sore, the gingivitis and stomatitis, evident. The gums were much swollen and fiery red, bleeding readily on manipulation. At points on the gums and cheeks were shallow ulcers with a white surface. Vincent's organisms were discovered in dark field examinations of smears taken from the gums and the ulcerations.

The anterior cervical lymph glands on both sides, the sub-lingual, the sub-maxilliary and the peritonsillar glands were slightly but definitely enlarged and tender.

On 17, May, the condition was unchanged. The blood examination was as follows: Red blood corpuscles, 5,650,000 per cubic millimeter. Haemoglobin, 88 per cent (Dare). Colourless corpuscles, 4,200 per cubic millimeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	5
Polymorphonuclear eosinophiles	16
Polymorphonuclear basophiles	3
"Mast cells"	2
Small lymphocytes	12
Large lymphocytes	23
Abnormal lymphocytes	3
Large mononuclear and transitional forms	16
Lymphoblasts	2
Myelocytes	11
Myeloblasts	1
Giant polymorphonuclears	4
Plasma cells	1
Megacaryocytes	1

On the following day, 18, May, the patient was much worse. He was drowsy and listless, and complained of aching "all over." He was unable to eat because of the intensely sore mouth. The temperature, for the first time, became definitely elevated; 101.5°F. (mouth). At no time were there chills or definite chilly sensations. The blood examination was as follows: Red blood corpuscles, 5,720,000 per cubic millimeters. Haemoglobin, 88 per cent (Dare). Colourless corpuscles, 4,500 per cubic millimeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	0.5
Polymorphonuclear eosinophiles	3
Polymorphonuclear basophiles	1
Small lymphocytes	13.5
Large lymphocytes	2.5
Large mononuclears and transitional forms	68
Myelocytes	5
"Smudges"	6.5

On 19, May, eight days after the onset of the "attack," he felt better, but was still weak, and complained of the sore mouth. The temperature was 100.4°F.

Examination of the blood: Red blood corpuscles, 5,790,000 per cubic millimeter. Haemoglobin, 75 per cent (Dare). Colourless corpuscles, 4,950 per cubic mililmeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	6
Polymorphonuclear eosinophiles	6
Polymorphonuclear basophiles	1
Small lymphocytes	24.5
Large lymphocytes	2
Large mononuclear and transitional forms	51
Myelocytes	7
"Smudges"	2.5

On 20, May, he felt that his "attack" was over. He was much improved symptomatically, the soreness of the mouth very much less annoying. The swelling and redness of the gums and buccal mucous membranes had diminished. The shallow ulcerations were healing. Examinations on the dark field of smears obtained from the affected areas showed fewer Vincent's organisms. The blood picture was essentially as on the preceding day.

On 21, May, the temperature was normal and the patient was free from unpleasant symptoms. Red blood corpuscles, 5,390,000 per cubic millimeter. Haemoglobin, 88 per cent (Dare). Colourless corpuscles were 5,500 per cubic millimeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	25
Polymorphonuclear eosinophiles	13
Polymorphonuclear basophiles	3
"Mast cells"	1
Giant polymorphonuclears	3
Small lymphocytes	21
Large lymphocytes	9
Abnormal lymphocytes	5
Large mononuclears and transitional forms	14
Myelocytes	6

On 23, May, he was feeling well, and the sore mouth was healing rapidly. Five days later the red blood corpuscles were 5,100,000 per cubic millimeter, haemoglobin, 88 per cent. (Dare), and the colourless corpuscles, 5,850 per cubic millimeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	46
Polymorphonuclear eosinophiles	5
Polymorphonuclear basophiles	2
"Mast cells"	1
Small lymphocytes	26
Large lymphocytes	8
Abnormal lymphocytes	2
Large mononuclear and transitional forms	5
Giant polymorphonuclear neutrophiles	2
Plasma cells	2
Myelocytes	1

Examination of the mouth showed only the residual gingivitis.

On 2, June, the patient was quite well. The blood picture on this date was as follows: Red blood corpuscles, 5,970,000 per cubic millimeter. Haemoglobin, 88 per cent (Dare). Colourless corpuscles, 5,600 per cubic millimeter.

Differential count

	per cent
Polymorphonuclear neutrophiles	52
Polymorphonuclear eosinophiles	5
Polymorphonuclear basophiles	2
Small lymphocytes	17
Large lymphocytes	12
Abnormal lymphocytes	3
Large mononuclear and transitional forms	7
Myelocytes	1

The colourless blood corpuscles were studied by one of us (Hansen) with a supravital staining method, at frequent intervals, especially during an "attack." It was noticed that during the time in which the granulocytes became reduced in number, that is, just before and during an "attack," the motility of the granulocytes became very much reduced and their viability, as well as their capacity to take the neutral red dye, very much diminished. The viability of the granulocytes was frequently no more than half of the normally expected time of life. The eosinophilic granulocytes, during the period of depression of the granular elements, showed, on the contrary, great motility and increased viability.

This course of events was repeated, so far as we could determine, with each attack. The changes in the blood are set forth graphically in Chart II.

The platelets, normal in number when first counted, appeared in general to be rather diminished during the attacks. In our specimens of 7, May, in an interval between attacks, and on 19, May, when the patient was beginning to come out of an attack, the platelets, as emphasized by Dr. Minot, were increased, very large and coarsely granular. Unfortunately regularly repeated counts of the platelets such as were made of the blood and the various varieties of leucocytes were not recorded.

On 14, June, 1928, at our suggestion the patient consulted Dr. George R. Minot of Boston. Dr. Minot confirmed our studies on previous preparations and wrote as follows of the appearances on 14, June.

"On June 14, 1928, the hemoglobin was 75 per cent and the red blood cell count was 5,060,000. The red cells appeared essentially normal. There was trivial achromia and rarely an elongated oval-ended red cell was seen. The cells averaged in diameter slightly smaller than normal.

"The blood platelets occurred in normal numbers and their histological character was normal.

"The blood clotted in normal time. The clot retracted promptly and normally and the serum contained no excess of bile pigments.

"The bleeding time was normal.

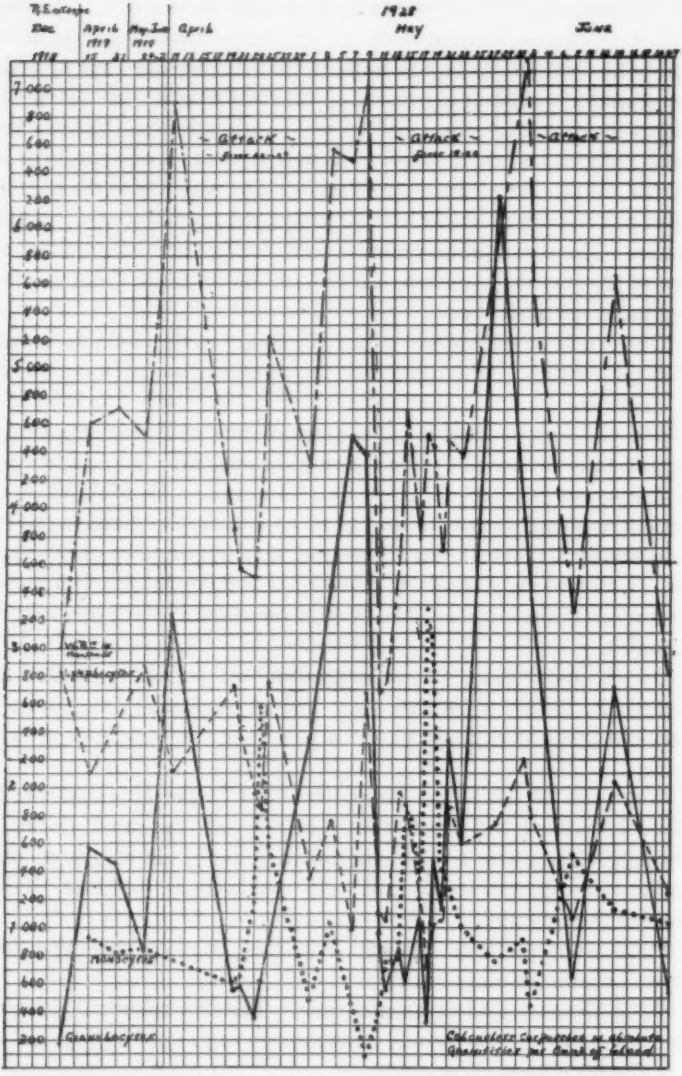

CHART II. SHOWING COLOURLESS CORPUSCLES IN ABSOLUTE QUANTITIES PER CUBIC CENTIMETER OF BLOOD

"The white cell count was 5,650. The differential count, as follows:

	per cent
Polymorphonuclear neutrophiles	29
Polymorphonuclear eosinophiles	15
Polymorphonuclear basophiles	4
Lymphocytes, normal small	29.5
Lymphocytes, normal large	6
Monocytes	1.5

"Cells somewhat larger than polynuclears, each with an irregular coarse large nucleus, with cytoplasm of frayed consistency, suggesting poorly developed cells, or cells "about to die," 20 per cent. These cells are probably of lymphoid or endothelial origin; 'atypical monocytes of some sort.' They were not very immature cells as nucleoli were not visible.

"Similar cells, smaller than polynuclear neutrophiles, with deep blue nucleus and fragile cytoplasm; atypical lymphocytic cells, 5 per cent.

"In fresh preparations, the motility of these monocytic cells seemed less than normal.

"The eosinophilia and basophilia is a peculiar feature, and although I can write a list of possible reasons I cannot give any adequate explanation. Their occurrence suggests, however, that the marrow itself may not be as much involved as is suggested by the low polynuclear count. The number of platelets and the lack of anemia gives one the same impression. In typical agranulocytic angina, I think, an absence of reduction of eosinophiles and basophiles, even after attacks, is apt to be a feature."

Additional comments of Dr. Minot on the specimens taken on 7, May, and 19, May, are worth noting. Fully 6 per cent of the cells in the specimens of 7, May, he regarded as atypical monocytes. He noted that the cells were torn and lacking a clean-cut morphological picture. Neutrophiles appeared "poorly formed" or "*lacking in vitality*"; "their cytoplasm wavy or smudgy in appearance. . . . ." On 19, May: "The atypical monocytic cells were 64 per cent. Forty-four per cent were cells half again as large as polynuclears, their cytoplasm filled with coarse, irregularly distributed granules. Each had a definite nucleus but this stained poorly and showed no nucleoli. Fourteen per cent were similar cells but their size was smaller than that of polynuclears. Ten per cent were cells about the size of poly-

nuclears, each with a two-lobed nucleus, and the cell was filled with brownish-staining granules. These atypical monocytic cells were like those on June 14 but perhaps more atypical, particularly as this preparation of May 19 showed the cells with the peculiar brownish granules."

Dr. Minot had seen instances of recurrent agranulocytic angina with as many as four attacks; never more. He had never seen a cyclic agranulocytosis.

The similarity of this periodicity to the menstrual cycle impressed him and he raised the question as to whether there could be some alteration of glands of internal secretion which had led to a cyclic depression of the bone marrow which lowered the resistance to infection. He had seen women with intermittent thrombopenic purpura which was apt to be associated with the menstrual period. Some of these had, in older literature, been described as vicarious menstruation. Could there be any cyclic change in pituitary or testicular function?

The childish voice, the sparse hair on the face, suggest some altered development. While skeptical as to the result, he suggested liberal amounts of whole pituitary gland or extract of anterior lobe.

He also suggested a liberal allowance of Vitamin B in the diet and, perhaps, liver feeding as well as exposure to the sun.

RECAPITULATION OF THE MAIN FEATURES OF THE CASE

1. At the age of two and a half months he suffered from attacks of furunculosis associated with leukopenia and a relative lymphocytosis.

2. From early childhood he has had "attacks" characterized by gastro-intestinal disturbances, listlessness, drowsiness and irritability, slight fever, sore mouth and a certain degree of cervical adenitis.

3. These attacks recur periodically every three weeks and last from six to ten days.

4. The fever is irregular, gradually rising to a fastigium on the fourth or fifth day and then disappearing by lysis in from four to six days.

5. Just before the attack and during the attack there is a leukopenia with pronounced agranulocytosis.

6. The eosinophilic leukocytes have always been somewhat increased in number, but especially so during an "attack," when the

neutrophilic leukocytes disappear almost completely from the circulating blood.

7. Early in the attack, when the granulocytes become greatly reduced in number, there is an increase in lymphocytes. There seems to be an increased activity of the lymphocytic system as exemplified by the appearance of immature lymphocytes and abnormal lymphocytes in the circulating blood.

8. At the height of the attack, a large number of large mononuclear and transitional cells are present in the circulating blood. These cells show active motility, are phagocytic and are of normal viability. They appear following a fall in the number of lymphocytes.

9. During the last two days of the illness, immature myeloid cells appear. These are forerunners of the returning adult, granular forms.

10. The hematopoetic system, so far as the red corpuscles are concerned, seems to be unaffected. The number of red corpuscles per cubic millimeter and the haemoglobin content have remained essentially normal throughout.

11. The platelets appear to be reduced in number during an "attack." At times between attacks they were numerous. No haemorrhagic manifestations have been noticed.

12. The motility and viability of the neutrophilic granulocytes is considerably reduced just before and during one of these periodical seizures. The motility of the granulocytes in the interim between "attacks" has never approached the normal. The eosinophilic granulocytes are perhaps the most actively motile, especially during an "attack," and show the greatest viability.

13. The outstanding and commonest physical manifestations during an "attack" are a sore mouth with swelling and reddening of the gums, with shallow ulcerations and induration of the gums, lips, tongue and buccal membranes, associated with a moderate degree of regional adenitis. From these sources Vincent's organisms have been obtained in large numbers.

14. The only abnormal physical manifestation observed between "attacks" is the chronic infection of the gums.

15. Treatment of the infection of the mouth has resulted in the disappearance of the excess of Vincent's organisms in the intervals, but

has not affected the periodical recurrences of fever and agranulocytosis nor the stomatitis which recurs with them. During the height of the "attack" these organisms are present in large numbers, but diminish greatly in quantity towards the end of the "attack."

16. Between "attacks" the patient seems to be in good health, and the blood shows no abnormalities save for the sluggish granulocytes.

Summary. In summary then, the patient, a man, 19 years of age, has been under the observation of various physicians since infancy. At the age of two and a half months he suffered from recurrent attacks of furunculosis associated with fever, leukopenia and lymphocytosis. After a course of autogenous vaccines, the furunculosis disappeared, but the child continued to have periods of poor health with fever, gastro-intestinal disturbances and a sore mouth. When eleven years of age he entered the Johns Hopkins Hospital where he was studied during two of his periodical "attacks" which have continued to the present day. A characteristic "attack" is as follows:—He becomes listless and fatigued; the complexion has been described as "yellowish." The temperature gradually rises, the maximum varying from 101° to 105°F., reaching its fastigium on the fourth day. Following this it falls gradually and irregularly to normal during the next 4 or 5 days. The cervical glands swell; the gums become red and puffy and are covered with ulcers, shallow at first, sometimes becoming deep and indurated. These appear also on lips, tongue and buccal surfaces. They gradually heal after the temperature falls. During the attack the patient is very irritable and fretful and occasionally rather drowsy. The appetite is poor. Nausea and vomiting are not uncommon.

This condition has persisted essentially until the present time. He is small in stature but well built. Beyond the circumstance that hair on the face is rather scanty and that the voice shows the changes common at puberty, physical examination reveals no striking abnormality, save a chronic infection of the gums, a pericementitis, and slight enlargement of the cervical and epitrochlear lymph glands.

The morphological changes in the blood during and between "attacks" follow a cyclical course. One or two days before the symptoms appear, there is a leukopenia at the expense of the granulocytic elements. There has been no anaemia. Frequently, at the height of

an attack, the adult neutrophilic granulocytes nearly disappear from the circulating blood, whereas the eosinophiles and lymphocytes are increased in number, relatively and absolutely. Following this, large mononuclear cells appear in great numbers in the circulating blood, the symptoms becoming accentuated. A few days later, neutrophilic granulocytes begin to reappear, heralded by immature myeloid cells. With this the large mononuclear cells gradually diminish to normal proportions. Study of the blood with supravital stains reveals a striking reduction in the motility and viability of the neutrophilic granulocytes preceding and during the attack. The diminished resistance of the neutrophilic granulocytes persists to a certain extent between attacks. The vital resistance of the eosinophiles, on the other hand, is normal or increased. Usually, at the end of ten days, the morphology of the circulating blood is essentially normal, save for a persistent slight eosinophilia. This series of events has been observed repeatedly. Between attacks the patient is essentially well.

This is a very remarkable picture rather suggestive of a periodical intoxication. What the cause of this may be is by no means clear. One might fancy that it is a result of some cyclic infection or, on the other hand, that it is related to the setting free of some toxic substance in association with the premature lysis of inherently defective cells.

If the former be the case, one naturally turns to the one obvious point of pathological change, that in the mouth, as a possible source of intoxication. To this end we have made every effort to institute thorough treatment of the gingivitis. This has been extremely difficult to accomplish owing to the unwillingness of the patient to follow advice.

On the other hand, however, it cannot be denied that stomatitis may in some patients, be associated secondarily with changes in the blood. We are unaware of any recognized instance of cyclic Vincent's angina or of cyclic agranulocytosis. The association of agranulocytosis with stomatitis is, however, an interesting and recognized clinical incident. In some diseases of the blood such as leukaemia or aplastic anaemia, in which there may be agranulocytosis, a grave stomatitis or, at least, a tendency to buccal infection is not uncommon. That agranulocytosis may occur without stomatitis is true, and, in this

patient's history, there is a record of attacks unassociated with sore mouth.

If conditions associated with agranulocytosis increase the susceptibility to buccal infection, then the mouth of him who has a well marked pyorrhoea or of him who neglects the care of his gums would naturally be more vulnerable than that of him who has a clean mouth. Our main attention, therefore, was directed to the care of the mouth, and the patient was put under the direction of Doctor Coriell who made every effort to improve the condition of the gums.

It was, however, impossible to obtain the full coöperation of the patient who was not to be induced to take the necessary care of his mouth. Never-the-less, the condition of the gums was considerably improved and the teeth remain in better condition a year later. We had hoped to be able to carry out studies of the toxicity of the serum in the intervals and during the attacks, and further to investigate the relative vulnerability of the corpuscles especially as compared with those of other human beings. Observations by one of us (Hansen) shortly before the onset of an attack, during a period of agranulocytosis, showed that normal leukocytes of the same blood group mixed with the patient's serum lost their motility and became rounded in forty-five minutes while several of the previously active motile granulocytes were dissolved. This same serum had no influence on washed lymphocytes or monocytes. In a control mixture of type II serum and homologous washed leukocytes the granulocytes retained their motility for an hour and a half. Unfortunately these studies could not be continued.

Our main effort, as has been said, was therefore directed towards the improvement of the condition of the mouth in the hope that by increasing the resistance of the buccal mucosa, we might at least remove one of the gravest symptoms, and perhaps break a vicious circle.

Extract of the anterior lobe of the pituitary body 0.325 t.i.d. for a week; thereafter 0.75 t.i.d. was ordered.

It was the good fortune of one of us (Thayer) to see the patient again three months ago. He had been able to pass satisfactorily through about one-half of the curriculum of the freshman year in an University, despite the persistence of his attacks. While it has been impossible to induce the patient to take proper care of his teeth, the

condition of the gums was better. The voice while still "changing," was distinctly more mature. The patient seemed stronger and better. Though the attacks continue, one gains the impression that he is improving and is able to lead a more normal existence.

He was then under the observation of Dr. Bloomfield of San Francisco and Dr. Rhodes of Berkeley at whose request an interesting bacteriological study of the mouth was made by Professor T. D. Beckwith of the University of California. Cultures were taken by aspiration from within the gums under intensely inflamed areas, and the following organisms were obtained:

> "A. A micrococcus, which is haemolytic and which ferments glucose, sucrose and maltose. This is not a Streptococcus and frankly I am unable to find any description of the organism within the literature.
> "B. Streptococcus salivarius . . . .
> "C. Neisseris perflava . . . . "

Professor Beckwith was desirous of making massive blood cultures between attacks and at the height of his severe attacks, which we hope may be accomplished. He further suggested a carefully determined acid-base balance at the same periods.

CONCLUSIONS

We are presenting a remarkable instance of cyclic, agranulocytic angina associated with fever and constitutional symptoms but without anaemia, beginning at the age of two and one-half months, and recurring at intervals of approximately three weeks, during the entire life of a man 20 years of age. While apparently unique in medical literature, the picture is so sharply defined and clear that we are inclined to think that it represents a definite complex of symptoms which may be less infrequent than one might fancy at the moment. For this reason, we desire to place the history on record.

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BULLETIN
OF
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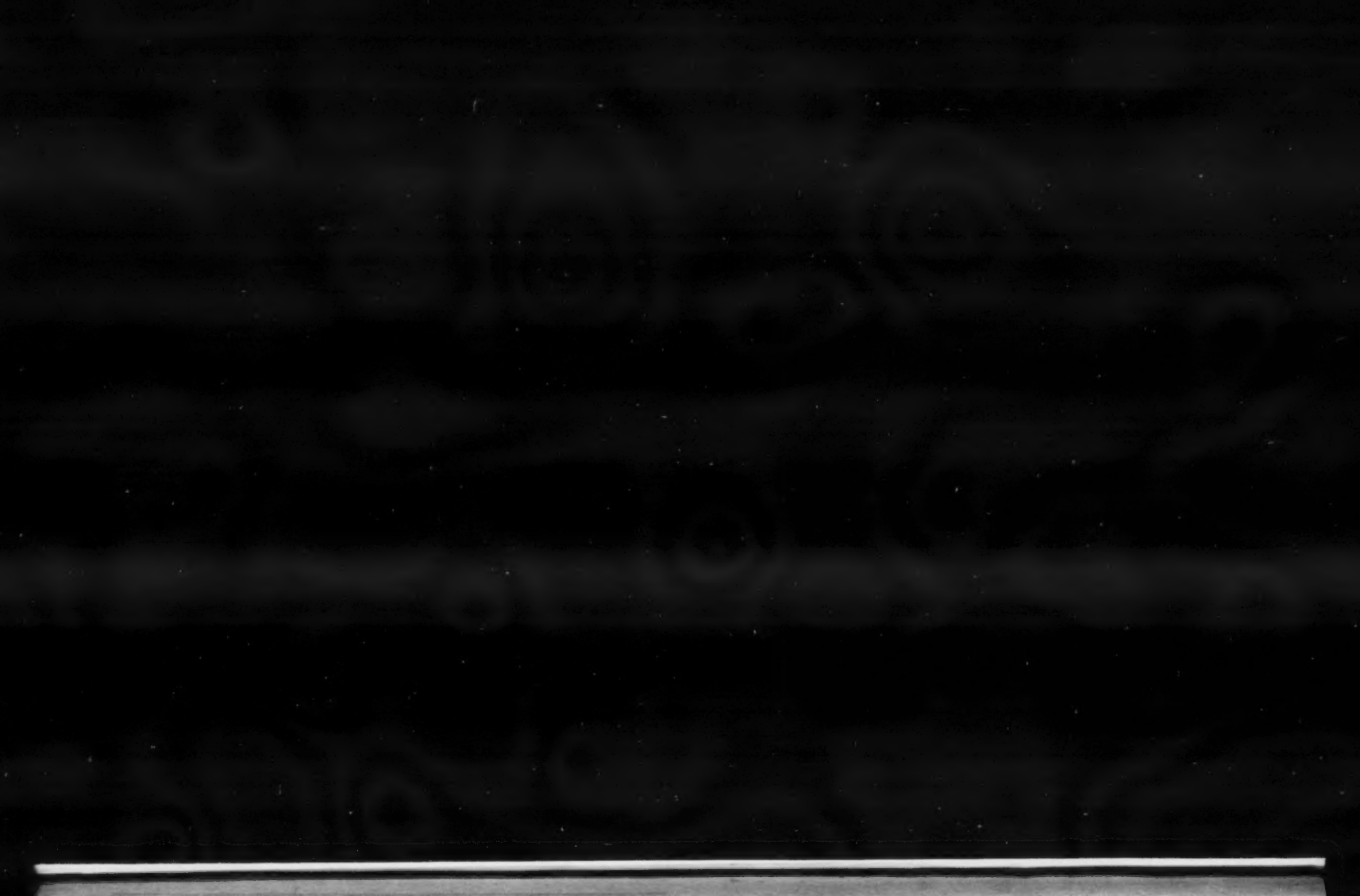
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